ASSOCIATION OF SUGAR INTAKE AND INFLAMMATION IN NEWLY DIAGNOSED COLORECTAL CANCER PATIENTS

by

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A thesis submitted to the faculty of
The University of Utah
in partial fulfillment of the requirements for the degree of

Master of Science

Department of Nutrition and Integrative Physiology

The University of Utah

August 2017

The University of Utah Graduate School

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ABSTRACT

Colorectal cancer (CRC) is a global health problem. In the United States, approximately 1 in 20 people will be diagnosed with CRC in their lifetime. Typically, CRC is associated with inflammation. Many factors influence CRC risk and development, most of which work through an inflammation connection. While genetics can play a role in CRC risk, lifestyle factors that promote inflammation seem to largely influence CRC risk and development. CRC inflammation factors mainly result from diet. Cancer is consistently linked with inflammation. Furthermore, increased systemic inflammation, as evaluated by chemokines, is consistently related to increased recurrence and decreased survival of CRC. The purpose of this study is to assess sugar intake (specifically fructose, glucose, and sucrose) and its association with systemic inflammatory and angiogenesis markers in colorectal cancer patients. ColoCare is a multicenter, international prospective cohort study with the goal of following over 5,000 patients who are newly diagnosed with CRC. The study employs longitudinal assessment of biomarkers and health behaviors and repeated sampling at multiple time points of multiple biological specimens. Specifically, the blood samples analyzed for this paper were from baseline blood samples collected pre- or peri-surgery. At 6-months post-baseline, the EPIC food frequency questionnaire (FFQ) was administered. This food frequency questionnaire covered the year prior, which means it assessed diet 6-months pre-diagnosis and the 6-months post-diagnosis. To evaluate

inflammation, we measured blood plasma inflammatory markers associated with cancer: C-reactive protein (CRP), serum amyloid A (SAA), intercellular adhesion molecule (SICAM), vascular cell adhesion molecule (SVCAM), interleukin-6 (IL-6), interleukin-8 (IL-8), monocyte chemoattractant protein 1 (MCP1), and tumor necrosis factor α (TNFα) and blood plasma angiogenesis markers associated with cancer: vascular endothelial growth factor-a (VEGFA) and vascular endothelial growth factor–d (VEGFD). Overall, no significant associations between sugar intake and systemic inflammatory and angiogenesis markers were observed. With body mass index (BMI(kg/m^2)) stratification, there were significant inverse correlations between sugar intake and VEGFD and MCP1 levels in those with a BMI < $25kg/m^2$ and a significant positive association between sugar intake and SVCAM in those with a BMI ≥ $25kg/m^2$, as measured by Pearson's correlation. Additionally, there was a significant association between higher sugar intake, as a percentage of total kcals, and lower VEGFD levels in those with a BMI < $25kg/m^2$, as measured by Spearman's correlation. These results are contrary to the popular slogan "sugar feeds cancer." Many cancer patients wish to use diet to improve their prognosis, yet research is lacking in this field. This study helps to improve the understanding between diet and colorectal cancer; further research is warranted.

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ACKNOWLEDGEMENTS

I would like to thank my committee members, Dr. Kristine Jordan, Dr. Julie Metos, Dr. Maureen Murtaugh and Dr. Neli Ulrich, for their support in completing this thesis project. I would also like to thank Dr. Jürgen Böhm for his guidance as well as Tengda Lin for his statistical expertise.

BACKGROUND AND LITERATURE REVIEW

1.1 Colorectal Cancer Prevalence and Incidence

Colorectal cancer (CRC) is a global health problem. In the United States, approximately 1 in 20 people will be diagnosed with CRC in their lifetime.[1] CRC is the fourth most common cancer in the United States, comprising 8.0% of all new cancer cases.[1] Despite improving survival rates, CRC is the second leading cause of cancer-related mortality in the United States.[1, 8] In Europe, CRC is the second most common cancer and the second most common cause of cancer-related deaths.[9] Additionally, CRC incidence continues to increase in both men and women, with a high variation in incidence rates across Europe.[10] Globally, cancer rates continue to rise, with unequal distributions of diagnosis and mortality between developed and less developed nations.[11, 12] Overall, these statistics highlight the burden that CRC exerts on society worldwide.

1.2.1 Mechanisms of CRC Development

CRC is a slow-growing, heterogeneous oncological disease that develops via several pathways.[13, 14] For all pathways, genetic instability results from an accumulation of genetic and epigenetic molecular alterations.[15] Alterations occur in a stepwise fashion wherein normal mucosal cells transform into malignant cells.[16] Most CRC cancers originate as polyps which progress to adenomatous polyps and finally to malignant polyps, though only a small percentage of polyps become malignant.[17, 18] CRC is unique in that 75% of cases

develop sporadically, with only 25% of cases resulting from hereditary mutations.[17] Three main pathways to CRC exist: chromosomal instability, microsatellite instability, and CpG island methylator phenotype.[13, 15, 19-21] Chromosomal instability refers to the development of adenomas, and consequently cancer, through a series of progressive mutations of several genes, such as K-ras, APC, and p53.[22, 23] Chromosomal instability is the most common pathway to CRC and is often termed the "adenoma-carcinoma sequence."[24]

Portions of DNA contain "motifs" of repeated tracts of nucleotides, such as the sequence TATATATATA; "microsatellites" is the term for motifs that are repeated three to hundreds of times.[22] Microsatellite instability occurs when mismatch repair genes are inactivated and there is a change in the number of repeated DNA nucleotides ("motifs") in a microsatellite.[22] Finally, CpG islands are regions of DNA where there is a high frequency of cytosine nucleotides followed by guanine nucleotides. The cytosine nucleotides can be methylated or unmethylated, either of which can change the expression of a gene.[25] Most often, the cytosine nucleotide lacks methylation.[25] The CpG methylator phenotype pathway involves hypermethylation of CpG islands, which silences tumor suppressor genes. To note, all three of these pathways can occur in both sporadic and hereditary CRC.[20, 22]

1.2.2 CRC Risk Factors and the Role of Inflammation

Typically, CRC is associated with inflammation. Many factors influence CRC risk and development, most of which work through an inflammation connection.[2] While genetics can play a role in CRC risk, lifestyle factors that promote inflammation seem to largely influence CRC risk and development.[3] CRC inflammation factors mainly result from diet.[3]

Under normal conditions, colonocytes utilize short chain fatty acids as the main source of fuel.[3] Short chain fatty acids result from the fermentation of dietary fibers. Diets low in dietary fiber decrease the preferred fuel source for colonocytes and thereby negatively alter colonocyte metabolism.[3] High levels of nitrogenous compounds within the colon induce inflammation and mucosal damage in the colon, thereby resulting in a high-risk luminal environment.[3] Poor diet, as defined by the low consumption of fruits, vegetables, whole grains, legumes, and high intake of processed grains and meat, can directly and indirectly affect inflammation in the colon and rectum. Directly, red and processed meat and animal fats can trigger an inflammatory response via the formation of N-nitroso compounds and expansion of pro-inflammatory microorganisms.[3] Indirectly, poor diet resulting in weight gain and obesity can favor a systemic pro-inflammatory state.[3, 26] In addition, tissue injury, as a result of mechanical, infectious, or chemical damage, causes a chronic inflammation response.[3] This chronic inflammatory response confers an oxidative and anaerobic microenvironment.[3] This microenvironment is associated with DNA damage and altered cellular mechanisms, ultimately resulting in a failure of normal mucosa repair and renewal.[19] Additionally, chronic inflammation seems to increase cancer predisposition[27] through activating signaling pathways that directly or indirectly modulate the epigenome, which then affects genes in GI homeostasis and repair.

1.2.3 The Role of Epigenetics in CRC Development

Epigenetic modifications are changes in gene expression not related to changes in primary gene nucleotide sequence. These modifications include DNA methylation, histone modification, nucleosome remodeling, chromatin modifications, and miRNA processing

and diversity.[28, 29] CRC occurrence is more often due to sporadic changes rather than genetic alterations. These sporadic changes seem to largely result from epigenetic modifications.[28]

Recent research suggests diet can drive epigenetic changes; this provides a molecular mechanism that may explain, in part, the connection between diet and CRC.[27, 30] Diet can render protective or inducive epigenetic modifications related to CRC.[30] Within CRC specifically, different epigenetic modifications have been found on different genes. Broadly speaking, epigenetic modifications contribute, determine, and affect CRC initiation, progression, and recurrence.[31] Fortunately, epigenetic modifications are reversible, so dietary changes may have a considerable impact on CRC occurrence and progression.

1.2.4 Markers to Measure Systemic Inflammation

Hanahan and Weinberg first proposed the hallmarks of cancer as the following: sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis.[32] Colotta suggested inflammation is the seventh hallmark of cancer.[33] Cancer is consistently linked with inflammation.[4] Furthermore, increased systemic inflammation, as evaluated by chemokines, is consistently related to increased recurrence and decreased survival of CRC.[4-7] The mechanism behind increased inflammation and increased recurrence and decreased survival of cancer is thought to relate to the tumor-promoting effects of inflammation in the tumor microenvironment.[4] Inflammation aids in proliferation and survival of malignant cells, promotes angiogenesis and metastasis, and undermines

immune responses.[4]

Chemokines fall in the family of chemotactic cytokines.[4] Cancer-related inflammation is largely regulated by chemokines and these chemokines regulate tumor growth, angiogenesis, and metastasis.[34] Broadly, across many cancers, CRP, TNFα, MCP1, IL-6, and IL-8 are connected with inflammation[34, 35]; ICAM and VCAM are associated with inflammation and cancer cell invasion[35]; VEGFA and VEGFD are correlated with cancer cell angiogenesis.[34, 35] In CRC specifically, IL-6 and TNFα have been implicated in the promotion of tumor growth, invasion, and metastasis.[5] In a cohort of stage IV CRC patients, Sharma found a positive correlation between IL-8 and VEGF with carcinoembryonic antigen, which is often used to track cancer growth.[5] Additionally, Di Caro reported high VEGF levels associated with increased risk of CRC recurrence and worsened survival among stage 0-IV CRC patients.[6]

1.3 Diet and CRC

1.3.1 Epidemiological Evidence in Diet and CRC

Diet and CRC Prevention and Progression

Consistently, research demonstrates that the development of CRC cancer is more closely linked to diet than many other types of cancer.[36] Most research agrees that consumption of fruits, vegetables, and whole grains has a protective effect in CRC prevention, whereas red meat and processed meat consumption has a detrimental effect.[37-39] Some evidence suggests that a high-fat diet can also promote CRC.[3, 40] In contrast to prevention, there is limited definitive research on how diet influences the progression of CRC cancer. Progression of CRC is defined as the cancer becoming more advanced and/or

spreading throughout the body. Regarding diet and CRC progression, the conclusions have been mixed. In newly diagnosed CRC patients, Zhu et al. reported decreased disease-free survival in patients (n=529) who consumed a high processed meat dietary pattern and this effect was elevated in women.[41] Additionally, no association between high sugar consumption or a prudent vegetable diet and mortality or recurrence was found. While this study did assess diet and CRC progression, the food frequency questionnaire (FFQ) only reflected diet the year prior to diagnosis.[41] In contrast, Fung et al. followed women (n=1201) who were diagnosed with CRC and evaluated diet through the Alternate Healthy Eating Index-2010 FFQ, which was administered 6-months after diagnosis.[42] The Alternate Healthy Eating Index-2010 awards higher scores for increased intake of foods associated with lower risk of chronic disease, such as fruits, vegetables, whole grains, and omega-3 fatty acids.[43] A higher Alternate Healthy Eating Index-2010 score was associated with lower overall mortality and when the components of the eating pattern were analyzed, decreased sugar-sweetened beverage and fruit juice consumption as well as decreased alcohol intake were specifically associated with lower overall mortality. Interestingly, no association with overall or CRC-specific mortality was found with the Western dietary pattern, which is characterized by higher intakes of red and processed meats, refined grains, sweets and desserts, and high fat dairy products.[42, 44] This pattern was also found in a study by Meyerhardt et al. that assessed dietary glycemic load and cancer recurrence among stage-III CRC patients[45]: a higher glycemic load and total carbohydrate intake were significantly associated with decreased disease-free, recurrence-free, and overall survival. Interestingly, the relationship between dietary glycemic load and disease-free survival was not linear; the relationship was stronger at higher quintiles of dietary glycemic load. Also,

the influence of glycemic load on survival was modified by body mass index (BMI(kg/m^2)): overweight or obese participants with a high glycemic load had a significantly worsened disease-free survival, as compared to healthy weight participants.[45] Fuchs et al. also found this progressive association between increased BMI(kg/m^2) and increased cancer recurrence among patients consuming sugar-sweetened beverages.[46] Lastly, Meyerhardt et al. noted glycemic load had a greater adverse influence on disease-free survival than the level of Western diet pattern.[45] Although available evidence at this point is limited, the majority of the evidence implicates dietary sugar as playing an adverse role in CRC progression and survival. The mechanisms through which sugar impacts CRC progression and outcome remain to be determined.

1.4 Sugar and Cancer

1.4.1 Definition of Sugars

The term sugar generally encompasses mono- and di-saccharides. The three mono-saccharides are glucose, fructose, and galactose. Sucrose, lactose, and maltose are the three di-saccharides. The most predominant sugars in the human diet are glucose, fructose, lactose, and sucrose.[47] Glucose is found in its polymer form within starch-rich foods such as grains.[47] Fructose is found naturally within fruit.[48] Fructose and glucose bond to form sucrose.[47] Sucrose is commonly referred to as "table sugar." Any prepared food product with "sugar" in the ingredients contains sucrose. Currently, a main source of sugar in human diets is high fructose corn syrup (commonly referred to as HFCS).[48] HFCS is a corn-derived sweetener developed in the 1960s.[48] Typically, HFCS is a mixture of 55% free fructose and 45% free glucose.[48] For example, a medium apple contains

approximately 19 grams of total sugar, with 11 grams as fructose.[49] A medium apple also contains 4.4 grams of fiber, which slows the rise in blood sugar.[47, 49] By comparison, a 12 oz can of Coke contains 39 grams of sugar in the form of HFCS, and no fiber.[50] Thus, one 12 oz can of Coke contains 21.45 grams of free fructose with no fiber to blunt the resulting rise in blood sugar.

Within the human body, only mono-saccharides are absorbed by intestinal mucosal cells utilizing different transporters, both active and facilitative, for each type. Once absorbed, fructose and galactose must pass through the portal vein to the liver for processing before reaching the general blood stream, whereas glucose can be absorbed throughout the body and thus does not require prior processing in the liver.[47] Fructose is of interest since its metabolism in the liver bypasses a regulatory step, unlike glucose and galactose.[48] By bypassing phosphofructokinase, a regulatory enzyme in glycolysis, high intake of fructose can result in dyslipidemia.[48] Fructose metabolism also occurs independently of insulin, unlike glucose. Additionally, fructose seems to induce hepatic and extrahepatic insulin resistance.[51] These metabolic findings support the role of fructose in obesity, blood lipid disorders, cardiovascular disease, and metabolic syndrome.[48]

1.4.2 Potential Role of Sugar in Cancer

The FDA defines "added sugar" as sugars that are added during the processing of foods or are packaged as "sugar."[52] Considerable epidemiological evidence associates added sugar with cardiovascular risk,[51, 53, 54] type II diabetes,[55] unfavorable lipid levels, insulin resistance, fatty liver, and metabolic syndrome.[51, 56] Currently, there is increasing evidence to suggest that sugar may play a role in cancer progression, through various mechanisms.[57]

Specifically, it appears sugar can help influence the cellular events within a tumor and affect the tumor microenvironment.[57] Furthermore, the type of sugar, fructose versus glucose, is a consideration. Fructose, in particular, increases reactive oxygen species, inflammation, and cytokines.[57] Research demonstrates there are fundamental differences in the way cancer cells utilize sugars, as compared to non-cancer cells.[57] For example, Zamora-León found the fructose transporter GLUT5 highly expressed in human breast cancer cells, but absent in normal human breast tissue.[58] However, sugar may confer different cancer risks depending on the tissue.[57] Overall, sugar appears to promote inflammation in a broad systemic context and specific tumor cell context.[57]

Broadly, it appears that some cancers are promoted by hyperinsulinemia and insulin resistance.[26, 57] Both hyperinsulinemia and insulin resistance are influenced by obesity and added sugar in the diet.[26, 57] The anabolic and anti-apoptotic effects of insulin are mediated by insulin binding to the insulin receptor or IGF-1 receptor.[26] The IGF-1 receptor exerts proliferative and anti-apoptotic effects within a cell. Increased insulin decreases IGFB-1&2 levels, which causes an increase in free IGF-1 availability.[26] Theoretically, chronic added sugar intake would result in overall increased insulin levels and thus tumor development would be promoted by the effects of elevated insulin and IGF-1.[26] Additionally, hyperglycemia alone leads to an increase in reactive oxygen species in B-cells and accumulation of advanced glycation end-products, which further increases inflammation and formation of reactive oxygen species. Collectively, these processes work to promote carcinogenesis.[26]

1.5 Hypothesis

Colorectal cancer patients with high added sugar (specifically fructose, glucose, and sucrose) intake, will have elevated circulating systemic inflammatory markers.

1.6 Primary Aim and Secondary Aims

1.6.1 Primary Aim

1. To assess the association between added sugar intake (specifically fructose, glucose, and sucrose) and circulating inflammatory markers in CRC patients. Potential confounders or effect modifiers include: age, gender, BMI(kg/m^2), kcal intake, Type II Diabetes Mellitus (T2DM), FFQ version, and diet change.

1.6.2 Secondary Aims

2. To assess the association between added sugar intake (specifically fructose, glucose, and sucrose) as a percentage of total kcal and circulating inflammatory markers in CRC patients. Potential confounders or effect modifiers include: age, gender, BMI(kg/m^2), kcal intake, T2DM, FFQ version, and diet change.
3. To assess the association between sugar intake *from sugar-sweetened beverages* and circulating inflammatory markers in CRC patients. Potential confounders or effect modifiers include: age, gender, BMI(kg/m^2), kcal intake, T2DM, FFQ version, and diet change.

METHODS

2.1 ColoCare Study Population

ColoCare is a multicenter, international prospective cohort study with the goal of following over 5,000 patients who are newly diagnosed with CRC. The seven study sites are the University Hospital in Heidelberg, Germany; the Fred Hutchinson Cancer Research Center in Seattle, WA; the Moffit Cancer Center in Tampa, FL; Cedars Mt. Sinai in Los Angeles, CA; Washington University School of Medicine in St. Louis, MO; the University of Tennessee in Memphis, TN; and the Huntsman Cancer Institute in Salt Lake City, UT. The overall aim is to develop evidence-based guidelines for physicians and patients to help increase survival of persons with CRC. To develop these guidelines, the study employed longitudinal assessment of biomarkers and health behaviors and repeated sampling at multiple time points of multiple biological specimens [Figure 1]. This study protocol included extensive follow-up observations for 60 months, with approval under the IRB File 310/2001-DACHS-ColoCare. Eligibility criteria were as follows: new diagnosis of colon or rectal or rectosigmoidal carcinoma; staged 0/I-IV (according to the American Joint Committee on Cancer based on histopathological findings); between 18-80 years of age; and able to provide informed consent. For this proposal specifically, the Heidelberg, Germany cohort data, with recruitment between February 2011 and October 2014, were analyzed. Specifically, patients with an available 6-month FFQ and baseline blood samples were included in the analysis.

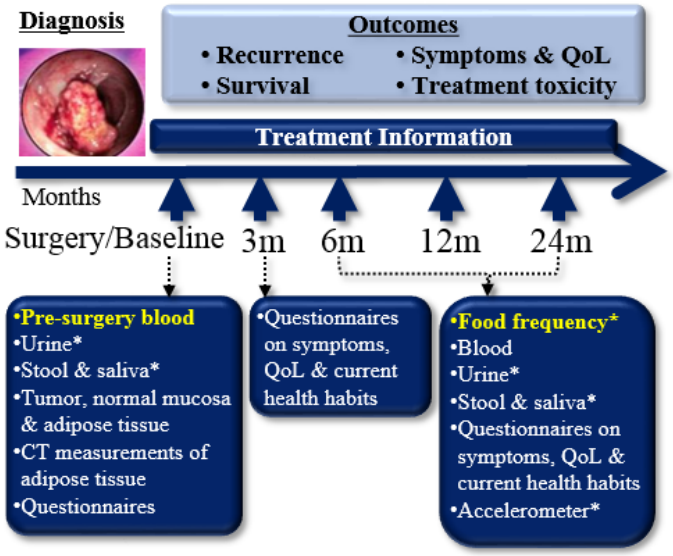

Figure 1: ColoCare Study Design.
In this project we utilized ColoCare blood collections at pre-surgery and FFQ at 6-months. The 6-month FFQ covered the previous 12-months: 6-months pre-diagnosis and 6-months post-diagnosis.

2.2 ColoCare Study Design

The design of the study is summarized in Figure 1. Participants were recruited at diagnosis of CRC (referred to as "baseline") and followed for 60 months. At baseline, pre-surgery blood was collected along with urine, stool, saliva, tumor, normal mucosa, and adipose tissue samples. The blood, urine, stool, and saliva specimen collections were repeated at 6, 12, and 24 months. Questionnaires covering health behaviors, symptoms, and quality of life were administered at baseline. These questionnaires were repeated at 3, 6, 12, 24, 36, 48, and 60 months. At 6-months post-baseline, the EPIC FFQ were administered. This FFQ covered the year prior, which means it assessed the 6-months pre-diagnosis and 6-months post-diagnosis. The blood samples used for this paper specifically

were from baseline blood samples collected pre- or peri-surgery. Finally, chart abstraction on treatment toxicity and recurrence was performed for 60 months. Outcome measures of the study (symptoms, quality of life, treatment toxicity, recurrence, and survival) were collected throughout the study.

2.2.1 Blood Sample Collection and Processing

Non-fasting blood samples were collected after diagnosis of colorectal cancer from ColoCare patients in Heidelberg, Germany. Blood samples were recollected at 6-, 12-, and 24-months post-baseline. As stated above, the blood samples used for this paper were the 6-month samples. Serum was extracted from the whole blood within 4 hours of the blood draw. Samples were then frozen at -80°C and shipped, on dry ice, to the Huntsman Cancer Institute (Salt Lake City, UT) for storage at -80°C until analysis.

2.2.2 Biomarkers of Inflammation and Angiogenesis

To evaluate inflammation, we measured blood plasma inflammatory markers associated with cancer: C-reactive protein (CRP), serum amyloid A (SAA), intercellular adhesion molecule (SICAM), vascular cell adhesion molecule (SVCAM), interleukin-6 (IL-6), interleukin-8 (IL-8), monocyte chemoattractant protein 1 (MCP1), and tumor necrosis factor α (TNFα) and blood plasma angiogenesis markers associated with cancer: vascular endothelial growth factor-a (VEGFA) and vascular endothelial growth factor-d (VEGFD).

2.2.3 Laboratory Methods (Meso Scale Discovery)

Serum samples were brought from -80°C to room temperature and pipetted into assay plates. A Meso Scale Discovery (MSD) sector 2400A (Meso Scale Diagnostics, Rockville, MD) was used to analyze the inflammation markers. The markers were split between plates as follows: IL-6, IL-8, MCP-1, and TNFα on a U-Plex plate; CRP, SAA, sICAM-1, and sVCAM-1 on a V-Plex Vascular Injury Plate; and VEGF-A and VEGF-D on a V-Plex Angiogenesis Panel 1 plate. Serum samples on the V-Plex plates had not been freeze-thawed. U-Plex plate serum was freeze-thawed twice. Freeze-thawing could have affected analyte levels; however, a literature review suggested the effect as negligible. Serum samples were all run in duplicate with three controls on each plate. The order of the samples was blinded and decoded after each assay run. The overall inter-plate coefficient of variability was 9.9% and intra-plate coefficient of variability was 4.6%.

2.2.4 Food Frequency Questionnaire

ColoCare Heidelberg utilizes the validated 148-item European Prospective Investigation into Cancer and Nutrition (EPIC) FFQ Potsdam, which is semi-quantitative and self-administered[59, 60]. The EPIC FFQ reflects the frequency of food intakes for all main food groups during the past 12 months. At the inception of the ColoCare project, the first version of the EPIC-FFQ was used; the second version (FFQ2) was introduced at the beginning of 2014. The second version includes more food options, regrouped food in some categories, added questions about general diet, and decreased monitoring supplement use from two years prior to one year prior. For this paper, FFQ data output spanned both versions of the FFQ.

A food database was used to evaluate the nutrients in each food item. Utilizing the European Prospective Investigation into Cancer and Nutrition (EPIC)-Soft software, food items were grouped into 80 classes.[61] According to the classification of the EPIC project, the 80 food classes were then assigned into 25 food groups based on nutrients or culinary usage. The food groups included four groups of vegetable intake, three groups of meat intake, one group of fruit and fish intake, four groups of fat intake, and twelve other food groups. Intake frequency was evaluated by a scale with values ranging from "never", "one time per month or less", "two to three times per month", "one to two times per week", to "three times per week or more." Portion sizes were defined by household measures. The food group data were combined with consumption frequency and typical portion sizes to yield estimates of kilocalorie (kcal) consumption and grams per day of fat, protein, carbohydrates, fiber, sugar, and micronutrients.

2.3 Statistical Methods

All data were analyzed in SAS (Version 9.4, SAS, Cary, NC). Descriptive statistics were used to summarize the distributions of the demographic clinical characteristics, nutritional intakes, and systemic inflammatory markers.

First, overall associations between sugar intake (both in terms of total kcal/day and percentage of kcal/day) and systemic inflammatory markers were evaluated. Additionally, correlation between BMI(kg/m^2) and sugar intake was assessed. Next, the data were stratified based on BMI(kg/m^2) (<25 and ≥ 25), age (<65 and ≥ 65), and sex. We then tested for correlation, utilizing both Pearson and Spearman's. Any associations were assessed visually to ensure they were driven by slopes, not outliers. We formally tested for effect

modification, based on the multiplicative rule. This formal test determined what variables were effect modifiers and what variables were confounders.

Then we ran several multiple linear regressions, controlling for various confounders while stratifying by BMI(kg/m^2). And we adjusted for total kcals two ways: first viewing fructose, glucose, and sucrose as a percentage of total kcals and second, controlling for total kcals as a confounder.

The analyzed data were collected between February 2011 and October 2014. The available sample size (ranging from only pre-surgical blood to both pre- and peri-surgical blood) was as follows: CRP: 138-153, SAA: 129-142, SICAM: 141-179, SVCAM: 141-157, VEGFA: 144-160, VEGFD: 144-161, IL-6: 150-169, IL-8: 150-169, MCP1: 150-169, and TNFα: 150-169.

A simple power analysis for a correlation between two variables, such as sugar level and an inflammation variable, indicated that correlations between 0.19 to 0.28 would have power levels from 0.7 to 0.9 for sample size ranging from 129-168 when using a significance level of .05.

RESULTS

3.1 Descriptive Statistics

Following previous research,[62-65] any patient with an estimated total kcal intake <600kcal/day and > 4000kcal/day was excluded from the final analysis (n=6). Further, one patient was excluded due to a data entry error, with a recorded intake of soda beyond allowable by the FFQ. Next, patients who were in an acute inflammatory reaction were excluded. Previous research utilized CRP outliers to detect an acute inflammatory reaction.[66] The following equations were used to determine CRP outliers above the 95th percentile: Caucasian or Hispanic men: age/5 = URL CRP in mg/L; Caucasian or Hispanic women: age/5 + 6 = URL CRP in mg/L; Black men: age/3 = URL CRP in mg/L; Black women: age/5 + 10 = URL CRP in mg/L. These equations resulted in an age-specific upper reference limit (URL) of CRP. This URL CRP was multiplied by 1.5 to yield approximately the 97th percentile of CRP. Any patient with CRP >50mg/L was dropped from all inflammatory markers except for VEGFD and VEGFA, which are angiogenesis markers (n=4). Any patient with a high CRP, as defined by 1.5 times the URL CRP, was removed from CRP and SAA analyses (n=11). Any patient with a CRP >1.5 times the URL CRP and a BMI of > 27.5 kg/m^2 was excluded from CRP and SAA analyses, due to overweightness explaining inflammation (n=1).

Study participants were both female and male. Participant ages ranged from 27 to 87 years old, with a mean age of 63 years. BMI ranged between 13.3 to 39.6 kg/m^2, with

a mean BMI of 26.3 kg/m^2. The cohort contained patients with all stages of CRC; however, the majority of patients were in Stage II and III. Among cohort patients, the site of cancer was split nearly evenly between the colon and rectum. A descriptive table of the characteristics of the cohort is displayed in Table 1.

Once outliers were dropped, all sugar distributions normalized. The distribution of intake, in kcals/day, of sucrose, glucose, and fructose varied greatly. As a percentage of total kcals, sucrose, glucose, and fructose intake ranged from 0-20%, 0-10%, and 0-20%, respectively. Total kcal intake displayed a normal distribution, with a mean of 2300 kcal/day.

The main contributors of sucrose in the diet were the "sugar and confectionary" food group, "dairy" food group, and "fruit" food group, respectively; these groups contributed 68% of total sucrose intake. The main contributors of glucose in the diet were the "fruit" food group, "non-alcoholic beverages" food group, and "sugar and confectionary" food group, respectively; these groups contributed 71% of total glucose intake. Finally, the main contributors of fructose in the diet were the "non-alcoholic beverages" food group, "fruit" food group, and "sugar and confectionary" food group, respectively, as 80% of total fructose intake.

Many of the systemic inflammatory markers exhibited long tails, as was expected. Once the inflammatory markers were log transformed, distribution normalized. The means and standard deviations of the markers are presented in Table 2.

Table 1: Descriptive statistics of study population (N=191)	
Demographics and clinical characteristics	
Variable	**n (%)**
Female	69 (36.1%)
Male	122 (63.9%)
Age (y)	
<50	20 (10.5%)
50-60	55 (28.8%)
>60	116 (60.7%)
BMI (kg/m^2)	
BMI <18.5	3 (1.6%)
BMI 18.5-24.9	60 (31.6%)
BMI 25.0-29.9	94 (49.5%)
BMI ≥ 30	33 (17.4%)
BMI unknown	1
Stage of CRC	
Stage 0	17 (8.9%)
Stage I	35 (18.3%)
Stage II	64 (33.5%)
Stage III	49 (25.7%)
Stage IV	26 (13.6%)
Site of CRC	
Colon	86 (45.0%)
Rectosigmoid	13 (6.8%)
Rectum	92 (48.2%)
Nutritional Intakes	**Mean ± S.D.**
Sugar intake in kcals/day	
Sucrose	255.8 ± 120.17
Glucose	85.0 ± 51.90
Fructose	109.6 ± 65.99
Sugar intake in percentage of kcals/day	
Sucrose/Total Kcals	10% ± 3%
Glucose/Total Kcals	0% ± 2%
Fructose/Total Kcals	0% ± 2%
Total kcals/day	
Total Kcals/day	2301 ± 673

Table 2: Descriptive statistics of study population (N=191)	
Biomarkers of inflammation and angiogenesis	
Biomarker (pg/ml)	**mean±S.D.**
CRP	4.4 ± 4
SAA	9.2 ± 15
SICAM	0.4 ± 0.16
SVCAM	0.6 ± 0.19
VEGFA	796.2 ± 589
VEGFD	856.9 ± 303
IL6	1.8 ± 10
IL8	26.9 ± 90
MCP1	184.4 ± 108
TNFa	1.2 ± 0.49

3.2 Analysis

Overall, no significant associations between sugar intake and systemic inflammatory and angiogenesis markers were observed. Notably, there was no correlation between sugar intake and BMI (kg/m^2). With BMI(kg/m^2) stratified, there were significant inverse correlations between sugar intake and VEGFD and MCP1 levels in those with a BMI < $25kg/m^2$ and a significant positive association between sugar intake and SVCAM in those with a BMI ≥ $25kg/m^2$, as measured by Pearson's correlation [Table 3]. With visual assessment, the associations reflected clear slopes and were not impacted by outliers [Figures 2 & 3]. When adjusted for age and gender, the significant associations between sugar intake and VEGFD and MCP1 were still present in those with a BMI < $25kg/m^2$, as was SVCAM in those with a BMI ≥ $25kg/m^2$ [Table 4].

Additionally, there were significant associations between sugar intake and VEGFD, MCP1, and IL-6 levels in those with a BMI < $25kg/m^2$ and significant associations between sugar intake and VEGFA and MCP1 in those with a BMI ≥ $25kg/m^2$, as measured by Spearman's correlation [Table 5]. Furthermore, when adjusted for age and gender, the significance as measured by Spearman's correlations between sugar intake and VEGFD, MCP1, and IL-6 was still present in those with a BMI < $25kg/m^2$, as was MCP1 in those with a BMI ≥ $25kg/m^2$ [Table 6].

Through testing for effect modification, we determined BMI(kg/m^2) to be an effect modifier and age and gender to be confounders. Next, we adjusted for total kcals two ways: 1) first viewing fructose, glucose, and sucrose as a percentage of total kcals and 2) controlling for total kcals as a confounder. The results were similar between the two models.

Table 3: Associations between sugar intake and systemic inflammatory and angiogenesis markers as measured by Pearson correlation and stratified by BMI(kg/m^2)

	BMI < 25kg/m^2					
Biomarker	*Sucrose (cal/day)*	*Glucose (cal/day)*	*Fructose (cal/day)*	*Sucrose/Total Kcals (%)*	*Glucose/Total Kcals (%)*	*Fructose/Total Kcals (%)*
log2-CRP	0 (1.00)	0.05 (0.73)	0.06 (0.69)	-0.06 (0.67)	-0.02 (0.92)	-0.03 (0.83)
log2-SAA	-0.14 (0.36)	-0.13 (0.38)	-0.11 (0.45)	-0.09 (0.55)	-0.11 (0.48)	-0.08 (0.58)
log2-SICAM	-0.15 (0.30)	-0.12 (0.42)	-0.1 (0.49)	-0.06 (0.68)	-0.01 (0.95)	0 (0.98)
log2-SVCAM	0.01 (0.92)	-0.02 (0.90)	0.03 (0.85)	0.03 (0.86)	-0.03 (0.81)	0.01 (0.95)
log2-VEGFA	0.03 (0.85)	-0.01 (0.94)	-0.02 (0.89)	0.05 (0.72)	-0.03 (0.82)	-0.03 (0.84)
log2-VEGFD	-0.38 (0.00) *	-0.42 (0.00) *	-0.41 (0.00) *	-0.25 (0.05)	-0.27 (0.04) *	-0.25 (0.05)
log2-IL6	-0.11 (0.43)	-0.22 (0.13)	-0.18 (0.21)	-0.11 (0.46)	-0.23 (0.10)	-0.19 (0.17)
log2-IL8	-0.06 (0.64)	-0.17 (0.19)	-0.15 (0.26)	0.06 (0.66)	-0.13 (0.31)	-0.1 (0.47)
log2-MCP1	-0.24 (0.07)	-0.27 (0.04) *	-0.27 (0.03) *	-0.25 (0.05)	-0.27 (0.04) *	-0.29 (0.02) *
log2-TNFa	-0.05 (0.74)	-0.06 (0.69)	0.02 (0.88)	0 (1.00)	-0.04 (0.80)	0.06 (0.68)

Table 3: Continued

BMI ≥ 25kg/m^2

Biomarker	*Sucrose (cal/day)*	*Glucose (cal/day)*	*Fructose (cal/day)*	*Sucrose/Total Kcals (%)*	*Glucose/Total Kcals (%)*	*Fructose/Total Kcals (%)*
log2-CRP	-0.03 (0.76)	-0.04 (0.68)	-0.12 (0.25)	0.17 (0.10)	0.08 (0.46)	-0.03 (0.79)
log2-SAA	-0.2 (0.06)	-0.16 (0.13)	-0.13 (0.21)	-0.08 (0.46)	-0.09 (0.42)	-0.06 (0.57)
log2-SICAM	-0.14 (0.17)	-0.1 (0.34)	-0.12 (0.24)	-0.03 (0.75)	-0.01 (0.89)	-0.07 (0.53)
log2-SVCAM	-0.01 (0.94)	0.2 (0.05) *	0.16 (0.12)	0.02 (0.87)	0.27 (0.01) *	0.21 (0.04) *
log2-VEGFA	-0.18 (0.07)	-0.14 (0.18)	-0.11 (0.26)	-0.06 (0.59)	-0.06 (0.53)	-0.03 (0.76)
log2-VEGFD	-0.05 (0.66)	0.08 (0.44)	0.08 (0.41)	-0.06 (0.53)	0.1 (0.34)	0.11 (0.30)
log2-IL6	0.06 (0.59)	0.17 (0.09)	0.09 (0.39)	0 (0.97)	0.12 (0.26)	0.03 (0.78)
log2-IL8	0.01 (0.94)	0.12 (0.22)	0.08 (0.42)	0.02 (0.83)	0.13 (0.18)	0.08 (0.43)
log2-MCP1	0.09 (0.36)	0.11 (0.27)	0.07 (0.44)	0.1 (0.32)	0.09 (0.33)	0.06 (0.53)
log2-TNFa	-0.09 (0.41)	0.03 (0.74)	0.03 (0.76)	-0.05 (0.66)	0.08 (0.43)	0.07 (0.52)

*Denotes significant correlation

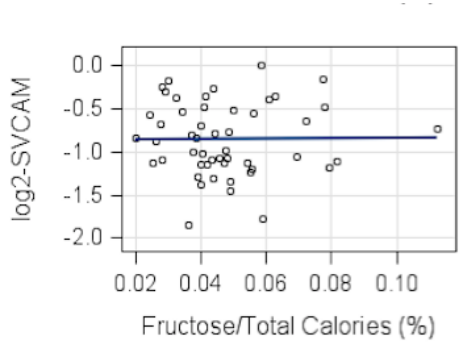

Figure 2: Association between percentage fructose intake and log2-SVCAM in patients with BMI < 25kg/m^2

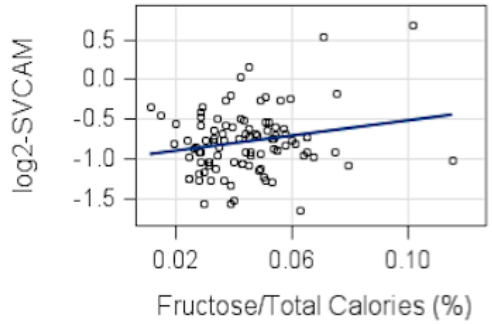

Figure 3: Association between percentage fructose intake and log2-SVCAM in patients with BMI ≥ 25kg/m^2

Table 4: Associations between sugar intake and systemic inflammatory and angiogenesis markers as measured by Pearson correlation, adjusted for age and gender and stratified by BMI(kg/m^2)

BMI < 25kg/m^2

Biomarker	*Sucrose (cal/day)*	*Glucose (cal/day)*	*Fructose (cal/day)*	*Sucrose/Total Kcals (%)*	*Glucose/Total Kcals (%)*	*Fructose/Total Kcals (%)*
log2-CRP	0 (0.98)	0.06 (0.70)	0.06 (0.68)	-0.06 (0.68)	-0.01 (0.95)	-0.03 (0.84)
log2-SAA	-0.13 (0.40)	-0.12 (0.43)	-0.11 (0.48)	-0.09 (0.55)	-0.1 (0.50)	-0.09 (0.57)
log2-SICAM	-0.13 (0.37)	-0.09 (0.55)	-0.08 (0.56)	-0.06 (0.69)	0.02 (0.92)	0.01 (0.95)
log2-SVCAM	0.05 (0.74)	0.05 (0.74)	0.06 (0.70)	0.04 (0.76)	0.04 (0.81)	0.04 (0.79)
log2-VEGFA	0.05 (0.71)	0.01 (0.95)	-0.01 (0.96)	0.06 (0.67)	-0.03 (0.82)	-0.04 (0.76)
log2-VEGFD	-0.37 (0.00) *	-0.42 (0.00) *	-0.4 (0.00) *	-0.26 (0.05) *	-0.31 (0.02) *	-0.29 (0.03) *
log2-IL6	-0.12 (0.41)	-0.2 (0.17)	-0.18 (0.20)	-0.08 (0.60)	-0.19 (0.19)	-0.18 (0.22)
log2-IL8	-0.05 (0.72)	-0.16 (0.22)	-0.14 (0.29)	0.06 (0.64)	-0.13 (0.33)	-0.1 (0.43)
log2-MCP1	-0.27 (0.04) *	-0.26 (0.05) *	-0.3 (0.02) *	-0.23 (0.08)	-0.22 (0.09)	-0.27 (0.04) *
log2-TNFa	-0.08 (0.61)	-0.06 (0.69)	0 (0.98)	0.01 (0.97)	-0.01 (0.93)	0.07 (0.64)

Table 4 Continued

BMI ≥ 25kg/m^2

Biomarker	*Sucrose (cal/day)*	*Glucose (cal/day)*	*Fructose (cal/day)*	*Sucrose/Total Kcals (%)*	*Glucose/Total Kcals (%)*	*Fructose/Total Kcals (%)*
log2-CRP	-0.01 (0.93)	-0.03 (0.81)	-0.12 (0.27)	0.18 (0.10)	0.08 (0.44)	-0.04 (0.73)
log2-SAA	-0.17 (0.13)	-0.13 (0.23)	-0.13 (0.23)	-0.08 (0.49)	-0.08 (0.47)	-0.08 (0.47)
log2-SICAM	-0.13 (0.21)	-0.09 (0.40)	-0.12 (0.26)	-0.04 (0.73)	-0.01 (0.89)	-0.07 (0.50)
log2-SVCAM	-0.01 (0.94)	0.20 (0.05)	0.16 (0.13)	0.03 (0.77)	0.29 (0.01) *	0.22 (0.04) *
log2-VEGFA	-0.16 (0.12)	-0.12 (0.24)	-0.10 (0.35)	-0.05 (0.61)	-0.07 (0.51)	-0.04 (0.73)
log2-VEGFD	-0.08 (0.46)	0.05 (0.60)	0.05 (0.65)	-0.06 (0.56)	0.10 (0.33)	0.09 (0.36)
log2-IL6	0.04 (0.68)	0.17 (0.10)	0.09 (0.39)	-0.01 (0.94)	0.12 (0.26)	0.04 (0.72)
log2-IL8	0.01 (0.89)	0.12 (0.23)	0.07 (0.47)	0.03 (0.77)	0.13 (0.19)	0.07 (0.48)
log2-MCP1	0.09 (0.37)	0.10 (0.30)	0.07 (0.48)	0.10 (0.30)	0.09 (0.33)	0.06 (0.54)
log2-TNFa	-0.07 (0.51)	0.03 (0.75)	0.02 (0.82)	-0.04 (0.69)	0.07 (0.50)	0.05 (0.64)

*Denotes significant correlations

Table 5: Associations between sugar intake and systemic inflammatory and angiogenesis markers as measured by Spearman correlation and stratified by BMI(kg/m^2)

BMI < 25kg/m^2

Biomarker	*Sucrose (cal/day)*	*Glucose (cal/day)*	*Fructose (cal/day)*	*Sucrose/Total Kcals (%)*	*Glucose/Total Kcals (%)*	*Fructose/Total Kcals (%)*
log2-CRP	-0.01 (0.97)	-0.06 (0.67)	-0.03 (0.85)	-0.06 (0.71)	-0.11 (0.46)	-0.11 (0.48)
log2-SAA	-0.13 (0.37)	-0.16 (0.28)	-0.15 (0.31)	-0.03 (0.83)	-0.07 (0.64)	-0.08 (0.60)
log2-SICAM	-0.11 (0.46)	-0.08 (0.55)	-0.09 (0.53)	-0.06 (0.66)	-0.03 (0.86)	-0.03 (0.83)
log2-SVCAM	-0.08 (0.56)	-0.09 (0.52)	-0.09 (0.51)	-0.07 (0.61)	-0.09 (0.52)	-0.05 (0.70)
log2-VEGFA	-0.13 (0.32)	-0.21 (0.11)	-0.22 (0.09)	0 (1.00)	-0.05 (0.70)	-0.05 (0.69)
log2-VEGFD	-0.3 (0.02) *	-0.39 (0.00) *	-0.38 (0.00) *	-0.09 (0.51)	-0.2 (0.13)	-0.18 (0.18)
log2-IL6	-0.28 (0.04) *	-0.29 (0.04) *	-0.25 (0.08)	-0.13 (0.36)	-0.12 (0.40)	-0.11 (0.45)
log2-IL8	-0.08 (0.52)	-0.2 (0.12)	-0.15 (0.26)	0.05 (0.73)	-0.11 (0.40)	-0.03 (0.83)
log2-MCP1	-0.3 (0.02) *	-0.38 (0.00) *	-0.36 (0.00) *	-0.34 (0.01) *	-0.33 (0.01) *	-0.32 (0.01) *
log2-TNFa	-0.13 (0.38)	-0.05 (0.74)	0 (0.99)	0 (0.99)	0.01 (0.93)	0.11 (0.46)

Table 5: Continued

BMI ≥ 25kg/m^2

Biomarker	*Sucrose (cal/day)*	*Glucose (cal/day)*	*Fructose (cal/day)*	*Sucrose/Total Kcals (%)*	*Glucose/Total Kcals (%)*	*Fructose/Total Kcals (%)*
log2-CRP	-0.02 (0.84)	-0.08 (0.45)	-0.14 (0.20)	0.19 (0.07)	0.13 (0.24)	0.03 (0.77)
log2-SAA	-0.18 (0.09)	-0.09 (0.38)	-0.08 (0.45)	-0.1 (0.34)	0.01 (0.92)	0.01 (0.95)
log2-SICAM	-0.09 (0.37)	-0.2 (0.05)	-0.19 (0.06)	0.06 (0.55)	-0.09 (0.38)	-0.12 (0.27)
log2-SVCAM	0.01 (0.94)	0.04 (0.70)	0.08 (0.45)	0.04 (0.69)	0.06 (0.53)	0.11 (0.28)
log2-VEGFA	-0.21 (0.03) *	-0.17 (0.10)	-0.17 (0.09)	-0.05 (0.61)	-0.01 (0.89)	-0.03 (0.73)
log2-VEGFD	-0.04 (0.68)	0 (0.96)	0.04 (0.69)	-0.02 (0.88)	0.02 (0.86)	0.02 (0.81)
log2-IL6	0.05 (0.61)	0.02 (0.85)	0.02 (0.88)	0.02 (0.84)	0.05 (0.66)	-0.01 (0.89)
log2-IL8	0.02 (0.87)	0.08 (0.41)	0.1 (0.31)	0.04 (0.69)	0.14 (0.13)	0.14 (0.14)
log2-MCP1	0.19 (0.04) *	0.18 (0.07)	0.15 (0.11)	0.11 (0.24)	0.18 (0.06)	0.13 (0.17)
log2-TNFa	-0.07 (0.51)	0.05 (0.60)	0.02 (0.81)	-0.05 (0.64)	0.08 (0.43)	0.06 (0.53)

*Denotes significant correlations

Table 6: Associations between sugar intake and systemic inflammatory and angiogenesis markers as measured by Spearman correlation, adjusted for age and gender and stratified by BMI(kg/m^2)

BMI < 25kg/m^2

Biomarker	*Sucrose (cal/day)*	*Glucose (cal/day)*	*Fructose (cal/day)*	*Sucrose/Total Kcals (%)*	*Glucose/Total Kcals (%)*	*Fructose/Total Kcals (%)*
log2-CRP	0.00 (1.00)	-0.06 (0.71)	-0.03 (0.87)	-0.05 (0.73)	-0.10 (0.51)	-0.10 (0.49)
log2-SAA	-0.13 (0.40)	-0.15 (0.32)	-0.15 (0.33)	-0.03 (0.86)	-0.05 (0.74)	-0.08 (0.62)
log2-SICAM	-0.08 (0.57)	-0.05 (0.73)	-0.07 (0.62)	-0.07 (0.65)	-0.01 (0.96)	-0.04 (0.78)
log2-SVCAM	-0.06 (0.70)	-0.05 (0.71)	-0.08 (0.57)	-0.07 (0.61)	-0.06 (0.68)	-0.07 (0.65)
log2-VEGFA	-0.11 (0.43)	-0.19 (0.15)	-0.21 (0.12)	0.00 (1.00)	-0.06 (0.68)	-0.07 (0.60)
log2-VEGFD	-0.27 (0.04) *	-0.35 (0.01) *	-0.34 (0.01) *	-0.13 (0.33)	-0.23 (0.08)	-0.23 (0.09)
log2-IL6	-0.30 (0.04) *	-0.33 (0.02) *	-0.28 (0.05) *	-0.11 (0.44)	-0.11 (0.44)	-0.11 (0.45)
log2-IL8	-0.05 (0.72)	-0.17 (0.20)	-0.13 (0.35)	0.04 (0.78)	-0.12 (0.36)	-0.06 (0.66)
log2-MCP1	-0.33 (0.01) *	-0.44 (0.00) *	-0.40 (0.00) *	-0.32 (0.01) *	-0.32 (0.01) *	-0.32 (0.01) *
log2-TNFa	-0.15 (0.34)	-0.08 (0.62)	-0.04 (0.79)	0.02 (0.89)	0.02 (0.88)	0.10 (0.51)

Table 6: Continued

BMI ≥ 25kg/m^2

Biomarker	*Sucrose (cal/day)*	*Glucose (cal/day)*	*Fructose (cal/day)*	*Sucrose/Total Kcals (%)*	*Glucose/Total Kcals (%)*	*Fructose/Total Kcals (%)*
log2-CRP	-0.01 (0.94)	-0.07 (0.53)	-0.13 (0.24)	0.20 (0.07)	0.13 (0.24)	0.03 (0.78)
log2-SAA	-0.14 (0.20)	-0.05 (0.67)	-0.05 (0.66)	-0.10 (0.38)	0.02 (0.88)	0.00 (0.98)
log2-SICAM	-0.07 (0.50)	-0.18 (0.09)	-0.18 (0.09)	0.07 (0.50)	-0.09 (0.42)	-0.12 (0.26)
log2-SVCAM	0.02 (0.85)	0.05 (0.65)	0.08 (0.47)	0.06 (0.56)	0.08 (0.47)	0.11 (0.29)
log2-VEGFA	-0.19 (0.07)	-0.14 (0.18)	-0.15 (0.15)	-0.05 (0.65)	-0.01 (0.94)	-0.04 (0.73)
log2-VEGFD	-0.07 (0.52)	-0.01 (0.90)	0.01 (0.91)	-0.01 (0.91)	0.03 (0.78)	0.02 (0.83)
log2-IL6	0.05 (0.62)	0.02 (0.86)	0.01 (0.91)	0.03 (0.78)	0.05 (0.61)	-0.01 (0.92)
log2-IL8	0.04 (0.68)	0.11 (0.25)	0.12 (0.24)	0.05 (0.61)	0.16 (0.10)	0.14 (0.14)
log2-MCP1	0.19 (0.05) *	0.17 (0.07)	0.15 (0.13)	0.12 (0.22)	0.18 (0.06)	0.13 (0.17)
log2-TNFa	-0.04 (0.67)	0.08 (0.43)	0.04 (0.69)	-0.03 (0.74)	0.10 (0.33)	0.07 (0.50)

*Denotes significant correlations

In the first multiple linear regression, three markers presented as significant when viewing fructose, glucose, and sucrose as a percentage of total kcals, while adjusting for gender and age and stratifying by BMI(kg/m^2). In patients with a BMI < $25kg/m^2$, as all types of sugar increased, VEGFD levels decreased. This trend also occurred with MCP1, yet only with fructose. In those with a BMI ≥ $25kg/m^2$, as glucose and fructose intake increased, SVCAM levels increased as well [Table 7].

In the second multiple linear regression, which controlled for total kcals, in those with a BMI < $25kg/m^2$, as glucose and fructose increased, VEGFD levels decreased. This negative trend did not occur with MCP1 in the model. In those with a BMI ≥ $25kg/m^2$, as glucose and fructose intake increased, SVCAM levels increased as well [refer to Table 8]. Within this particular model, the associations attenuated when adjusted for T2DM [Table 9].

Table 7: Multiple linear regression analysis between sugar intake and systemic inflammatory and angiogenesis markers, adjusted for age and gender and stratified by BMI(kg/m^2)

Note, kcal/day equates to per 1000kcals of specified sugar per day

BMI < 25kg/m^2

Coefficient of regression (pvalue)	*Sucrose (kcal/day)*	*Glucose (kcal/day)*	*Fructose (kcal/day)*	*Sucrose/Total Kcals (%)*	*Glucose/Total Kcals (%)*	*Fructose/Total Kcals (%)*
log2-CRP	0.06 (0.98)	1.63 (0.70)	1.67 (0.68)	-2.99 (0.68)	-0.97 (0.95)	-3.04 (0.84)
log2-SAA	-1.11 (0.40)	-2.46 (0.43)	-2.10 (0.48)	-3.16 (0.55)	-7.36 (0.50)	-6.28 (0.57)
log2-SICAM	-0.37 (0.37)	-0.59 (0.55)	-0.55 (0.56)	-0.65 (0.69)	0.36 (0.92)	0.21 (0.95)
log2-SVCAM	0.12 (0.74)	0.28 (0.74)	0.31 (0.70)	0.42 (0.76)	0.73 (0.81)	0.81 (0.79)
log2-VEGFA	0.33 (0.71)	0.16 (0.95)	-0.13 (0.96)	1.46 (0.67)	-2.44 (0.82)	-2.62 (0.76)
log2-VEGFD	-1.34 (0.00) *	-4.74 (0.00) *	-3.80 (0.00) *	-3.83 (0.05) *	-13.85 (0.02) *	-10.41 (0.03) *
log2-IL6	-1.12 (0.41)	-4.09 (0.17)	-3.68 (0.20)	-2.89 (0.60)	-13.70 (0.19)	-12.84 (0.22)
log2-IL8	-0.42 (0.72)	-3.42 (0.22)	-2.88 (0.29)	2.12 (0.64)	-9.73 (0.33)	-7.77 (0.43)
log2-MCP1	-1.17 (0.04) *	-2.74 (0.05) *	-3.01 (0.02) *	-3.86 (0.08)	-8.19 (0.09)	-10.08 (0.04) *
log2-TNFa	-0.39 (0.61)	-0.66 (0.69)	0.05 (0.98)	0.09 (0.97)	-0.46 (0.93)	2.34 (0.64)

Table 7: Continued

	BMI ≥ 25kg/m^2					
Coefficient of regression (pvalue)	*Sucrose (kcal/day)*	*Glucose (kcal/day)*	*Fructose (kcal/day)*	*Sucrose/Total Kcals (%)*	*Glucose/Total Kcals (%)*	*Fructose/Total Kcals (%)*
log2-CRP	-0.14 (0.93)	-0.94 (0.81)	-2.64 (0.27)	7.82 (0.10)	8.32 (0.44)	-2.53 (0.73)
log2-SAA	-2.41 (0.13)	-4.69 (0.23)	-2.86 (0.23)	-3.30 (0.49)	-7.79 (0.47)	-5.33 (0.47)
log2-SICAM	-0.60 (0.21)	-0.99 (0.40)	-0.82 (0.26)	-0.48 (0.73)	-0.44 (0.89)	-1.49 (0.50)
log2-SVCAM	-0.03 (0.94)	2.07 (0.05)	0.99 (0.13)	0.38 (0.77)	8.03 (0.01) *	4.14 (0.04) *
log2-VEGFA	-1.63 (0.12)	-2.62 (0.24)	-1.75 (0.35)	-1.59 (0.61)	-4.05 (0.51)	-1.96 (0.73)
log2-VEGFD	-0.37 (0.46)	0.55 (0.60)	0.40 (0.65)	-0.85 (0.56)	2.85 (0.33)	2.39 (0.36)
log2-IL6	0.46 (0.68)	3.75 (0.10)	1.36 (0.39)	-0.26 (0.94)	7.49 (0.26)	1.77 (0.72)
log2-IL8	0.21 (0.89)	3.87 (0.23)	1.59 (0.47)	1.32 (0.77)	11.96 (0.19)	4.83 (0.48)
log2-MCP1	0.59 (0.37)	1.46 (0.30)	0.67 (0.48)	2.07 (0.30)	3.84 (0.33)	1.82 (0.54)
log2-TNFa	-0.34 (0.51)	0.34 (0.75)	0.16 (0.82)	-0.64 (0.69)	2.03 (0.50)	1.05 (0.64)

*Denotes significant correlations

Table 8: Multiple linear regression analysis between sugar intake and systemic inflammatory and angiogenesis markers, adjusted for age, gender, and total kcals and stratified by BMI(kg/m^2)

Note, kcal/day equates to per 1000kcals of specified sugar per day

BMI < 25kg/m^2			
Coefficient of regression (pvalue)	*Sucrose (kcal/day)*	*Glucose (kcal/day)*	*Fructose (kcal/day)*
log2-CRP	-1.70 (0.55)	0.37 (0.95)	0.33 (0.95)
log2-SAA	0.21 (0.92)	-0.41 (0.92)	0.49 (0.90)
log2-SICAM	-0.30 (0.65)	-0.20 (0.88)	-0.10 (0.94)
log2-SVCAM	0.47 (0.40)	0.67 (0.53)	0.86 (0.44)
log2-VEGFA	1.41 (0.30)	1.47 (0.69)	0.82 (0.79)
log2-VEGFD	-1.26 (0.08)	-4.55 (0.02) *	-3.50 (0.04) *
log2-IL6	-0.43 (0.84)	-3.82 (0.31)	-3.45 (0.37)
log2-IL8	1.06 (0.56)	-2.84 (0.43)	-2.12 (0.56)
log2-MCP1	-0.93 (0.30)	-1.94 (0.26)	-2.56 (0.15)
log2-TNFa	0.60 (0.57)	0.60 (0.75)	1.94 (0.31)

Table 8: Continued

BMI ≥ 25kg/m^2			
Coefficient of regression (pvalue)	*Sucrose (kcal/day)*	*Glucose (kcal/day)*	*Fructose (kcal/day)*
log2-CRP	3.66 (0.06)	6.49 (0.16)	0.88 (0.75)
log2-SAA	-1.60 (0.43)	-2.18 (0.65)	-1.34 (0.64)
log2-SICAM	-0.12 (0.85)	0.31 (0.83)	-0.13 (0.88)
log2-SVCAM	0.19 (0.73)	3.57 (0.01) *	1.76 (0.03) *
log2-VEGFA	-1.04 (0.42)	-1.27 (0.62)	-0.28 (0.90)
log2-VEGFD	-0.24 (0.69)	1.20 (0.32)	1.08 (0.30)
log2-IL6	0.03 (0.98)	4.11 (0.13)	1.17 (0.54)
log2-IL8	0.27 (0.89)	5.11 (0.17)	2.15 (0.41)
log2-MCP1	0.74 (0.38)	1.65 (0.31)	0.70 (0.54)
log2-TNFa	-0.55 (0.41)	0.48 (0.70)	0.24 (0.78)

*Denotes significant correlations

Table 9: Multiple linear regression analysis between sugar intake and systemic inflammatory and angiogenesis markers, adjusted for age, gender, total kcals, and T2DM and stratified by BMI(kg/m^2)			
BMI < 25kg/m^2			
Coefficient of regression (pvalue)	*Sucrose (Kcal/day)*	*Glucose (Kcal/day)*	*Fructose (Kcal/day)*
log2-CRP	-1.57 (0.56)	0.55 (0.91)	0.65 (0.90)
log2-SAA	-0.05 (0.98)	-0.50 (0.89)	0.66 (0.86)
log2-SICAM	-0.40 (0.53)	-0.40 (0.74)	-0.42 (0.73)
log2-SVCAM	0.18 (0.75)	0.08 (0.94)	-0.16 (0.88)
log2-VEGFA	0.42 (0.77)	0.29 (0.94)	-0.46 (0.88)
log2-VEGFD	-1.11 (0.12)	-3.23 (0.07)	-2.38 (0.12)
log2-IL6	0.07 (0.98)	-3.43 (0.34)	-2.71 (0.45)
log2-IL8	0.53 (0.77)	-3.57 (0.30)	-3.35 (0.33)
log2-MCP1	-0.77 (0.38)	-1.49 (0.37)	-1.95 (0.23)
log2-TNFa	0.26 (0.79)	0.71 (0.68)	1.42 (0.39)

Table 9: Continued			
BMI ≥ 25kg/m^2			
Coefficient of regression (pvalue)	*Sucrose (Kcal/day)*	*Glucose (Kcal/day)*	*Fructose (Kcal/day)*
log2-CRP	3.10 (0.10)	8.39 (0.10)	1.19 (0.67)
log2-SAA	-1.94 (0.34)	-2.84 (0.61)	-1.19 (0.70)
log2-SICAM	-0.59 (0.29)	-1.56 (0.32)	-0.47 (0.59)
log2-SVCAM	-0.17 (0.75)	1.50 (0.29)	1.00 (0.21)
log2-VEGFA	-1.21 (0.27)	0.34 (0.89)	0.50 (0.80)
log2-VEGFD	-0.84 (0.18)	-0.61 (0.65)	-0.20 (0.86)
log2-IL6	-0.05 (0.97)	3.35 (0.31)	-0.07 (0.98)
log2-IL8	1.09 (0.54)	5.05 (0.18)	2.31 (0.36)
log2-MCP1	0.86 (0.29)	2.83 (0.11)	0.90 (0.44)
log2-TNFa	-0.68 (0.30)	0.10 (0.94)	0.21 (0.81)

DISCUSSION

To our knowledge, this is the first study to assess the association between sugar intake via a FFQ with systemic inflammation and angiogenesis biomarkers. Overall, there was no association between sucrose, fructose, or glucose intake and systemic inflammatory and angiogenesis markers. This result was contrary to our hypothesis.

In the context of previous research, our main finding fits into the varied picture of diet and CRC. Zhu et al. found no association between high sugar consumption and disease-free survival of CRC; however, the FFQ only assessed diet the year prior to diagnosis.[41] In contrast, Fung et al. found increased sugar-sweetened beverage intake to lower overall mortality among women with CRC.[42]

However, with BMI(kg/m2) stratification, associations between sugar intake and VEGFD, SVCAM, and MCP1 became apparent: those with a BMI < 25kg/m^2 had lower VEGFD and MCP1 levels with higher sugar consumption. Previous research by both Meyerhardt and Fuchs also found BMI to be an effect modifier; however, the study outcomes were sugar consumption and CRC recurrence, as compared to sugar intake and biomarkers in the current study.[45, 46] In both the Meyerhardt and Fuchs studies, increased sugar consumption was associated with increased CRC recurrence, with increasing BMI(kg/m^2) intensifying the effect. However, most previous research suggests increased sugar consumption increases systemic and tumor-specific inflammation, not angiogenesis.[57]

Regarding VEGF, this biomarker has been found to be elevated in those with T2DM and obese individuals.[68, 69] Within our cohort, only 17 patients had T2DM. When stratified by BMI < 25kg/m^2 and BMI ≥ 25kg/m^2, 8% of patients with a BMI < 25kg/m^2 had T2DM and 11% of patients with a BMI ≥ 25kg/m^2 had T2DM. This distribution seems unlikely to have skewed the results.

While VEGF is known as an angiogenesis marker, some research has associated increased VEGF levels with worsened CRC prognosis.[6] We speculate that in patients with a BMI < 25kg/m^2, increased sugar plays a protective role against cancer cachexia, which is associated with worsened CRC prognosis. In contrast, in patients with a BMI ≥ 25kg/m^2, increased sugar may act predominately to increase inflammation.

With regard to SVCAM, this biomarker is considered an indicator of CRC progression.[70, 71] Given that increased sugar intake is associated with increased inflammation and increased inflammation is associated with worsened CRC prognosis, it follows that increased sugar consumption increased SVCAM levels in patients with a BMI ≥ 25kg/m^2. Obesity is known to induce systemic inflammation; therefore, increased sugar intake may intensify this relationship. This explanation may account for why this trend was not seen in patients with a BMI < 25kg/m^2.

4.1 Strengths and Limitations

A major study strength is the novel correlation of FFQ data with blood plasma inflammatory and angiogenesis markers. To note, many studies have assessed FFQ and cancer recurrence. However, to our knowledge, no previous study has assessed the association of diet with a clinically relevant marker of CRC. Additionally, this study

assessed a set of ten different markers. The association of FFQ data with blood plasma markers provides insight into the mechanism through which diet influences cancer progression. A second strength of this study is the existence of multiple cohorts within the study, allowing the possibility to validate findings in another group of patients within ColoCare. Lastly, the sample size is large, with adequate effect sizes for analyses.

Regarding limitations, the FFQ methodology occurs random measurement errors; another limitation of FFQ methodology is recall bias. However, the proposed FFQ was validated against two 24-hour dietary recalls.[59] Despite the validity of the FFQ, there is potential for a change in diet after patient diagnosis. To note, the FFQ used in this study does address whether there was a change in diet post-diagnosis. Therefore, we were able to account for reported dietary changes in the statistical analysis. We recognize that other foods besides sugar can have pro-inflammatory properties.[72] Further research could address the potential association between pro-inflammatory foods and blood plasma inflammatory markers.

CONCLUSION

In summary, this study employed a novel approach of assessing the association of sugar intake patterns and their systemic inflammatory and angiogenesis markers in CRC patients. No association was found between sugar intake patterns and systemic inflammatory and angiogenesis markers. Stratification of the data by BMI(kg/m^2) did yield significant inverse correlations between sugar intake, as a percentage of total kcals, and VEGFD and MCP1 levels in those with a BMI < $25kg/m^2$ and a significant positive association between sugar intake and SVCAM in those with a BMI ≥ $25kg/m^2$, as measured by Pearson's correlation. "Sugar feeds cancer" is a common phrase used among cancer patients, despite the lack of evidence for the statement. This study helps to expand the scientific understanding of the role of diet in colorectal cancer patients. Further research on evidence-based guidelines on dietary intake recommendations in colorectal cancer is warranted.

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